

Serial No. 09/348,469

with a random gene trap vector comprising a DNA construct, wherein the DNA construct (i) lacks a promoter, and (ii) comprises the sequence:

5' X-A-P-B-Q-C-Y 3'

in which

- X comprises a splice acceptor sequence;  
Y comprises a polyadenylation signal;  
P is an internal ribosome entry site (IRES);  
Q is the heterologous gene sequence; and  
A, B and C are, separately, optional linker sequences.

23. A method according to Claim 22 wherein the heterologous gene coding sequence is randomly inserted into an endogenous gene so that transcription of the heterologous gene coding sequence is directed by the host regulatory elements of the endogenous gene.

24. A method according to Claim 22 in which the splice acceptor permits functional integration of the heterologous gene coding sequence into an intron sequence.

25. A method according to Claim 22 wherein the heterologous gene coding sequence is expressed in an animal cell.

26. A method according to Claim 22 further comprising the step of identifying cells expressing the heterologous gene coding sequence.

27. A method according to Claim 26 wherein the heterologous gene coding sequence also codes for a selectable marker, such as antibiotic resistance, and the method comprises selecting cells that express the selectable marker.

28. A cell comprising a heterologous gene coding sequence inserted by the method of Claim 22.

29. A descendant of a cell according to Claim 28, wherein the descendant has inherited the inserted heterologous gene coding sequence.

30. An animal comprising a heterologous gene coding sequence inserted by the method of Claim 22.

31. A descendant of an animal according to Claim 30, wherein the descendant has inherited the inserted heterologous gene coding sequence.

32. A DNA construct for randomly inserting a heterologous gene sequence into a host genome, said construct lacking a promoter and comprising the sequence:

5' X-A-P-B-Q-C-Y 3'

in which

- X comprises a splice acceptor sequence;  
Y comprises a polyadenylation signal;  
P is an internal ribosome entry site (IRES);  
Q is the heterologous gene sequence; and  
A, B and C are, separately, optional linker sequences.

Serial No. 09/348,469

33. A DNA construct according to Claim 32 in which the splice acceptor permits functional integration of the heterologous gene into an intron sequence.

*WLC*  
34. A DNA construct according to Claim 32 in which the heterologous gene sequence additionally codes for a selectable marker, such as antibiotic resistance, to facilitate selection of cells containing an heterologous gene has been inserted into an endogenous gene.

*B1*  
*Cond*  
35. A method of inserting a heterologous gene coding sequence into an endogenous gene in a eukaryotic cellular host cell genome and expressing said heterologous gene coding sequence, comprising the step of transforming the host cell with a random gene trap vector comprising a DNA construct, wherein the heterologous gene coding sequence (1) lacks a promoter, and (2) comprises the sequence:

5' X-A-P-B-Q-C-Y-Z 3'

in which

X comprises a splice acceptor ;

Y comprises a polyadenylation signal ;

P is an internal ribosome entry site (IRES);

Q is the heterologous gene sequence;

A, B and C are, separately, optional linker sequences; and

Z is a selectable marker cassette optionally adapted for

recombinatorial deletion following introduction of an X-A-P-B-Q-C-Y-Z construct in a gene which is not expressed in ES cells.

LAW OFFICES

FINNEGAN, HENDERSON,  
FARABOW, GARRETT,  
& DUNNER, L.L.P.  
1300 I STREET, N. W.  
WASHINGTON, D. C. 20005  
202-408-4000

Serial No. 09/348,469

36. A method according to Claim 35 wherein the heterologous gene coding sequence is inserted into an endogenous gene so that transcription of the heterologous gene coding sequence is directed by the host regulatory elements of the endogenous gene.

37. A method according to Claim 35 in which the splice acceptor permits functional integration of the heterologous gene coding sequence into an intron sequence.

38. A method according to Claim 35 wherein the heterologous gene coding sequence is expressed in an animal cell.

39. A method according to Claim 35 further comprising the step of identifying cells expressing the heterologous gene coding sequence.

40. A method according to Claim 36 wherein the heterologous gene coding sequence also codes for a selectable marker, such as antibiotic resistance, and the method comprises selecting cells that express the selectable marker.--

#### REMARKS

Prior to examination of this application, Applicants have canceled claims 1-21 and added new claims 22 - 40. The new claims are supported by the application and claims as filed and do not add new matter.